

THE EFFECT OF LOW TEMPERATURE
AND GIBBERELIC ACID ON THE AMYLOLYTIC ACTIVITY
AND GROWTH OF A TROPICAL GRASS
(DIGITARIA DECUMBENS STENT.)

By

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TABLE OF CONTENTS

	<u>Page</u>
ACKNOWLEDGMENTS	ii
LIST OF TABLES	iv
LIST OF FIGURES	v
ABSTRACT	vi
CHAPTER I	
INTRODUCTION AND GENERAL LITERATURE REVIEW	1
CHAPTER II	
AMYLOLYTIC ACTIVITY IN LEAVES OF TROPICAL AND TEMPERATE GRASSES	12
CHAPTER III	
REVERSAL OF LOW TEMPERATURE EFFECTS ON A TROPICAL PLANT BY GIBBERELIC ACID	33
CHAPTER IV	
LOW NIGHT TEMPERATURE, GROWTH AND AMYLOLYTIC ACTIVITY OF TWO SPECIES OF DIGITGRASS	42
CHAPTER V	
SUMMARY AND CONCLUSIONS	47
LITERATURE CITED	50
BIOGRAPHICAL SKETCH	59

LIST OF TABLES

<u>Table</u>	<u>Page</u>
1 Effect of Leaf Position on the Amylolytic Activities of Leaf Extracts of Five Weeks Regrowth of 'Pangola'	22
2 The Effect of Night Temperature Pretreatment and Incubation Temperatures on the Amylolytic Activities of Leaf Extracts (on Dry Weight Basis)	24
3 The Effect of Night Temperature Pretreatment and Incubation Temperatures on the Amylolytic Activities of Leaf Extracts (on Fresh Weight Basis)	26
4 Effect of Low Night Temperature and Gibberellic Acid on the Growth of 'Pangola'	39
5 Effect of Gibberellic Acid on Amylolytic Activity and Starch Content of 'Pangola' at Two Night Temperature Regimes	40
6 Effect of Gibberellic Acid on the <u>in vitro</u> Activity of the Starch-degrading Enzymes of 'Pangola'	41
7 The Effect of Night Temperature Pretreatment on the Amylolytic Activities of Leaf Extracts of Two Species of Digitgrass	45
8 The Effect of Various Temperature Regimes on the Growth of Two Species of Digitgrass	46

LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
1 Starch-degrading activity of crude extracts as shown by the starch-agar plate method.	28
2 The effect of low temperature on the amylolytic activity of 'Pangola' and orchardgrass on protein basis.	30
3 The effect of low temperature on the amylolytic activity of 'Pangola' and orchardgrass on DNA basis.	32

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The Effect of Low Temperature
and Gibberellic Acid on the Amylolytic Activity
and Growth of a Tropical Grass
(Digitaria decumbens Stent.)

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The potential for pasture and forage productivity of 'Pangola' (Digitaria decumbens Stent.) is markedly reduced by low night temperatures. Sterility and absence of variation make breeding and selection of 'Pangola' impossible. For this reason the biochemical mechanism for growth depression by low temperature and a method of reversing this effect were investigated. Growth, amylolytic activity, and the effect of gibberellic acid (GA_3) on growth and certain biochemical events in 'Pangola' at low temperatures were determined. Crude leaf extracts were used for enzyme assay. Amylolytic activity was measured spectrophotometrically as the disappearance of starch (starch-iodine complex). Enzyme activity varied greatly with the position of the leaves on the shoots. Young, fully expanded leaves near the shoot apices have the highest starch-degrading activity. This activity decreased in extracts of older leaves taken from progressively lower positions on the shoot.

Amylolytic activities of leaf extracts of 'Pangola' were compared to those of orchardgrass (Dactylis glomerata L.) subjected to the same treatments. Treatment of 'Pangola,' a tropical species, with 10C night temperature for 3 nights markedly reduced the starch-degrading enzyme activity of leaf extracts whereas this was not the case with orchardgrass, a temperate species. The growth of orchardgrass is little affected by 10C night treatments. Subjecting 'Pangola' to 10C night temperature resulted in higher leaf starch content and decreased amylolytic activities, dry weights, leaf areas and shoot lengths as compared with plants exposed to 30C night temperatures. Applications of 10^{-5} M gibberellic acid (GA_3) reversed these effects of 10C night temperature. The direct addition of GA_3 to the reaction mixture did not alter the in vitro amylolytic activity of crude leaf extracts of 'Pangola' during their incubation either at 10C or 30C. This indicates that the effect of low temperature was on synthesis of amylolytic enzymes.

A comparison was made on the growth and amylolytic activities of leaf extracts of 'Pangola' and Digitaria pentzii (P.I. 299753) at low temperatures. The D. pentzii exhibits better winter survival than does 'Pangola.' The reductions in the growth and amylolytic activity caused by low temperatures are comparable in the two digitgrass species.

Data reported in these investigations suggest that reduction of growth of 'Pangola' by suboptimal night temperatures is due to the depression of amylolytic activity. There was no relation between winter survival and amylolytic activity. Moreover, the data suggest

that treatment of 'Pangola' with GA₃ may extend pasture production later into the growing season. However, further studies are necessary before it can be recommended for commercial use.

CHAPTER I
INTRODUCTION AND GENERAL
LITERATURE REVIEW

Introduction

'Pangola' (Digitaria decumbens Stent., tribe Paniceae) is one of the most important pasture grasses in Florida. Over 500,000 acres of 'Pangola' are grown for pasture in the southeastern United States (46). The major areas of distribution are in tropical and subtropical regions. Under irrigation in the Mediterranean-like climate of southwest Australia, 'Pangola' substantially out-yielded legumes (70). 'Pangola,' a perennial and vegetatively propagated grass, is most suitable for use in comparatively intensive forage systems, where forage is both grazed and harvested as hay and/or silage. The productivity and feeding value of 'Pangola' make this versatile grass an important forage crop in its area of distribution (46). However, its potential for forage productivity is reduced by low night temperatures in many areas. Sterility and absence of variation make breeding and selection of 'Pangola' impossible as methods to improve adaptation of the plant.

This investigation was initiated to delineate the biochemical reasons for low temperature growth reduction. This knowledge is necessary for establishing practices which would extend the growing season of 'Pangola' in areas which experience growth reduction resulting

from low night temperatures late in the growing season. To this end a study was made of the effects of low night temperatures on 'Pangola' growth as well as on certain biochemical events. Further studies were conducted to seek a method of reversal of the effect of low temperature.

General Literature Review

The internal plant cell environment is constantly influenced and modified by its surroundings. Temperature is one of the most critical factors in the environment and exerts a profound effect on all physiological activities of the cell by controlling, among other things, the rates of chemical reactions. Since plant species differ in their tolerance to temperature extremes, some species are injured by extreme temperature while other species remain unharmed. An injury to a specific species may be caused not only by the magnitude of the variation of the temperature from an optimal value, but also by how rapidly the temperatures fluctuate as well as the length of time the temperature remains at an extreme (84).

The effects of temperature extremes on many responses of higher plants have been investigated and reviewed. Bonner (17) used the term "climatic lesion" to designate a depression in growth caused by temperatures higher or lower than the optima for a particular plant. Plant growth includes increases in volume and dry weight (18). These are basically increases in energy found in chemical form. Growth differences reflect changes either in the photosynthetic activity of the leaf or in the rate of expansion or relative size of that photosynthetic organ (29).

The final product of photosynthesis in the chloroplast of many higher plants is assimilatory starch (3, 67). Starch accumulation occurs during light periods, and during dark periods, the starch

may be degraded to soluble sugars and translocated to other areas of the plant to serve as respiratory substrate or in the synthesis of other organic compounds (40, 41).

The level of atmospheric CO_2 fixed by tropical grasses is approximately twice as great as that of temperate grasses (33). The photosynthetic efficiency of tropical grasses makes them important for increasing total forage productivity. Thus research in methods to extend the distribution of these grasses into marginal areas has become increasingly important.

Role of Temperature in the Growth of Higher Plants

Growth responses of tropical and temperate grasses to temperature are considerably different. Many plants indigenous to tropical and subtropical areas of the world are physiologically injured when subjected to low (1 to 10C) temperatures (59). Subtropical and tropical grasses have high optimum temperatures for growth. Many species grow extremely slowly, if at all, at temperatures around 10 to 15C. In many tropical species the net photosynthetic rate remains low until 15C, and then increases rapidly up to a temperature of 35C or a little above. Temperate grasses vary little in growth response over a broad temperature range, and optimum temperatures are around 20C (29).

In rice, the reported minimum temperature for germination ranges from 9 to 14C (12). Christiansen and Thomas (27) and Christiansen (26) observed reductions in plant height, fiber quality, and delayed fruiting that were directly correlated to the length of exposure of the cotton plant to cold. The duration of 10C chilling period on germinating cotton seed is additive in inhibiting subsequent seedling growth

at favorable temperatures. Root elongation in grasses differs in response to low temperatures. Harris (44) showed that the roots of Bromus tectorum grow well at temperatures as low as 3C, while Agropyron spicatum roots grow very slowly at temperatures below 8 to 10C. Beevers and Cooper (9) compared growth of ryegrass seedlings at 12C and 25C. The lower temperature reduced the relative growth rate and doubled the sugar content as a result of reduced utilization of assimilate in respiration and new growth.

Reduction of growth at low night temperatures has been reported for Sorghum halepense (48), Bouteloua gracilis, Panicum virgatum (10), and Cynodon dactylon (103). A drop in night temperature from 25C to 10C reduced the growth rate of Paspalum dilatatum and Eragrostis curvula (80). Different responses have been demonstrated in tropical species. Whiteman et al. (99) showed the optimum temperatures for growth of Saccharum spontaneum to be 25C, of Saccharum robustum to be 30C, and of Saccharum officinarum to be 35C.

In contrast to the tropical grasses, the temperate grasses, such as Dactylis glomerata (34), Poa pratensis (76), Agrostis tenuis (63, 76), and Phleum pratense (76), have optimal temperatures ranging from 20C to 25C. The growth rate drops rapidly below 10C, but there is still some growth at 5C, and the plant remains healthy. Growth is reduced above 25C and may cease above 30C to 35C. Brown and Blazer (20) found that yields of orchardgrass (Dactylis glomerata L.) at 24C days and 18C nights were twice those of a 35C-day/29C-night regime.

In temperate grasses, like tropical grasses, contrasting climatic ecotypes show differential growth rates under both low and high temperatures; a good example is Dactylis glomerata. It has been shown that the effect of temperature on the growth of orchardgrass varies according to climatic origin of the population (32, 83).

Temperature effects on growth differ in different plant species. Benedict (10) showed a decrease in growth of both top and root of Bouteloua gracilis when the day temperature was 24C and the night temperature was 15C, while Agropyron smithii showed the opposite effect compared to those grown at 24C day and night temperatures. Chloroplast formation may be inhibited by low temperature, as tomatoes in a cold room at 8C have degenerate chloroplasts and abnormal grana (72). Cold treatment of 13C on Sorghum bicolor L. produced much more pigment than that of 23C treatment (75). It has been shown that mitotic cycle is temperature dependent (18,85); lower or higher than normal temperatures depress the mitotic division.

Temperature influences tillering, and optimum temperature for growth varies with the species (62). Tillering of ryegrass is increased by reducing night temperatures (4), while tillering of tall fescue is greatest when the grass is exposed to low temperatures during light periods (81).

Effect of Low Temperature on Translocation and Carbohydrate Metabolism

The transport of organic compounds from leaves to heterotrophic cells which constitute metabolic sinks is essential to the growth of higher plants. The first responses of the plant to low

temperature are decreased molecular and metabolic activities, and the result of these is suppression of growth (84).

Evidence has been offered to support the hypothesis that translocation of assimilate from a leaf is dependent on factors within the leaf itself, as well as on the growth activity and assimilate utilization in other parts of the plant (89). The rate of translocation of assimilate from the leaves of sugar cane plants is more dependent on root temperature than shoot temperature (21). Vernon and Arnoff (87), working with soybeans, and Hartt (43), working with sugar cane, found that reduction in growth is accompanied by a reduction in the rate of assimilate translocation at low temperatures. A low temperature jacket around the hypocotyl of bean plants reduced sugar movement from the roots to the leaf immediately above the jacket (15). A fair correlation between the temperature and the rate of translocation has been shown in tomato (94).

Webb (92) studied the translocation of sugar in the primary leaf blades of Cucurbita melopepo at temperatures ranging from 0 to 55C and found that below 10C and at 55C (and presumably above) there was an appreciable decrease in net assimilation rate; at 15C, movement of assimilate began to become restricted. Webb and Gorham (93), working with primary nodes of squash, found a zero rate of translocation at 0C or 55C and the highest rate at 25C. Geiger (39) demonstrated that little translocation occurred when the translocation path of sugar beet plant was subjected to 2-5C temperature treatments, relative to translocation rates at 28C to 30C.

Temperature has a large influence on the amount of organic acids and sugars in the plant (56). There is evidence that accumulation of assimilates in a leaf causes a decrease in the rate of assimilation in that leaf. In 1868, Boussingault proposed that the accumu-

lation of assimilates in an illuminated leaf may be responsible for a change in the net photosynthetic rate of that leaf (8). Kostytschew et al. (52) stated that midday depression of photosynthesis is caused by assimilate accumulation in the leaves. In an excellent review article, Neales and Incoll (65) reported that leaf assimilate levels may control the rates of photosynthesis and growth.

Curtis (30) demonstrated that lowering petiole temperature of bean plants to between 1C and 4-6C increased the carbohydrate content of the leaves and effectively retarded translocation. Burt (23) lowered the net leaf assimilation rate of potato plants by reducing the air temperature around the potato tubers. Thorn and Evan (82), Humpheries (47) and Burt (22) suggest that temperature may partially govern the photosynthetic rate of the plant by varying the ability of the plant to utilize the assimilate.

Warren(90,91) concluded that the depression of net assimilation rates in arctic regions is due to low temperatures which cause sugars to accumulate to levels at which they depress assimilation. Cell membrane permeability changes and lateral release of assimilates could explain some of the temperature effects on translocation (89).

Amylolytic Activity in Plants

Amylases are found in almost all plant cells. Alpha-amylase, a calcium-requiring enzyme (104), attacks starch randomly at $\alpha(1-4)$ linkages, and beta-amylase hydrolizes starch, starting at the non-reducing ends of the chain, splitting off beta-maltose by breaking the $\alpha(1-4)$ linkage (78). Together they liberate a mixture of reducing sugars. Both enzymes attack amylose and amylopectin. In

the presence of inorganic phosphate, phosphorylase breaks down starch and liberates glucose-1-phosphate from non-reducing ends of starch molecules (3).

In 1890, Haberlandt (42) observed the liquefaction of the starchy endosperm portion of rye seeds and the dissolution of starch grains by substances produced in the aleurone layers. It is now established that alpha-amylase is the chief enzyme involved in the initial degradation of starch in storage tissues to more soluble forms (11, 79), while phosphorylase and beta-amylase assist in the further conversion of these forms to free sugar (11). Also, phosphorylase (31), alpha-amylase (38), and beta-amylase (40, 41) are reported to be responsible for degradation of starch in the leaf plastids. Changes in the environment of these enzymes will alter their behaviors and functions.

It is often found that at temperatures well below optimum, Arrhenius' equation (plotting the log of velocity against the reciprocal of absolute temperature) no longer applies. However, an apparent activation energy higher than expected occurs at these temperatures (74). Resistance to this type of low temperature inhibition might be effected by an increased production of the rate-limiting enzyme and/or a change in the level of activation energy required. Walker and Hope (88) showed that as the temperature is increased, in addition to the expected activation of alpha-amylase, an effect was noted in the hydrolysis of raw starch granules. They worked with saliva and crystalline salivary alpha-amylase at low temperatures and showed that the extent of adsorption to starch granules increased at low temperatures and higher pH's. If conditions

unfavorable for the adsorption of the enzyme were chosen, the rate of hydrolysis of starch granules was increased. According to Badenhuisen (7) spaces between starch molecules are different in starch granules from various sources. Therefore, the action of amylolytic enzymes may take place as exocorrosion or endocorrosion, e.g., acting on the outside of the starch granules or penetrating into the interior of the granules, depending on the nature of the starch molecules. Thus the substrate can be an important factor for determining amylolytic enzyme activity. Large differences have been shown in the required activation energies for the same enzyme when it is isolated from different sources. Activation energy levels (in K cal/mole) reported for alpha-amylase are as follows: 7.0 in Hordeum, 10.5 in Aspergillus, and 13.5 in Sus sp. (36).

Effect of Growth Regulators on the Amylolytic Activity and Growth of Plants

The causes of low temperature lesions are still not known for certain, but a theoretical basis exists as a result of recent studies on enzyme behavior at low temperatures (57). It has been stated that depression of growth by low temperature may be overcome by production of a higher concentration of the rate-limiting enzyme(s). Ketellapper (54) demonstrated that the adverse effects of unfavorable temperature on plant growth can be partially or completely prevented by application of essential metabolites to plants growing in such unfavorable conditions. The nature of the effective metabolites depended on the plant species involved and the temperature at which it was growing. When thiamin was applied to Cosmos at temperature

regimes of 17C days and 10C nights or of 20C days and 14C nights, a 30% increase in dry weight resulted (16, 53). Application of a 10% sucrose solution to pea plants grown at 23C days and 17C nights caused a 56% increase in dry weight bringing the dry weight up to the level of plants grown under optimal conditions of 17C days and 17C nights (53).

Attempts to increase the yield of annual crops such as Triticum vulgare and Zea mays by application of gibberellin have been unsuccessful (2, 64). In contrast, it has been shown that Poa sp. and Cynodon dactylon responded more to gibberellin at low temperatures (101). The most pronounced effect of gibberellic acid is modification of plant growth. Gibberellic acid (GA) has been shown to increase alpha-amylase activity in barley endosperm (28, 35, 49, 55, 66, 86, 102). Protein and ribonucleic acid can be regulated by GA (37). Studies (25) with aleurone cells of barley indicate that GA_3 enhances the incorporation of ^{14}C -uridine and ^{14}C -adenosine into RNA, and the authors concluded that gibberellin stimulates de nova synthesis of alpha-amylase by controlling the synthesis of mRNA. GA accelerated growth and amylase activity in germinating barley seed; this effect is inhibited by chloramphenicol sodium succinate, indicating that alpha-amylase is synthesized during germination and that GA accelerates this synthesis (77).

Application of exogenous GA_3 to a clone of Trifolium pratense caused the formation of two new protein peaks four and fifteen days after the treatment (50). Treatment of pea plants with GA_3 markedly increased protein synthesis (19). Blacklow and McGuire (13) have shown that a foliar spray of 1% GA increased foliage growth of tall

fescue during the winter. GA enhanced tomato fruit ripening at both higher and lower than optimal temperatures (1). Extension of wheat-leaf sections by GA_3 application has been shown (68).

It seems that the endogenous level of GA_3 or a block in the utilization of GA_3 would determine the effect of applied GA_3 . Radley (69), working with tall and dwarf wheat, found that application of GA_3 to tall wheat cultivars markedly stimulated the growth of tall seedlings but not the growth of dwarf seedlings. GA_3 increased the amount of soluble carbohydrates in the leaves of the tall cultivars but not in those of the dwarfs. A higher amount of endogenous GA_3 occurred in the germinating grains, light-grown seedlings, and developing stems of dwarf cultivars than in those of the tall cultivars.

In seeking a solution, first the effect of low temperatures on the starch-degrading enzymes of temperate and tropical plants was investigated. These two types of plants have different growth optima regarding temperature. Therefore, a comparative study was made on the effect of temperature on the amylolytic activities of crude leaf extracts. Comparisons were made between tropical 'Pangola' (Digitaria decumbens Stent.) and temperate orchardgrass (Dactylis glomerata).

CHAPTER II
AMYLOLYTIC ACTIVITY IN LEAVES OF
TROPICAL AND TEMPERATE GRASSES

Introduction

Growth and photosynthesis of 'Pangola' (Digitaria decumbens Stent.), a tropical grass, are reduced by night temperature of 10C (14, 61, 95, 96, 97). Studies of leaf ultrastructure, as well as chemical analyses, showed that illumination of the plants for 12 hr at 30C resulted in the accumulation of large amounts of starch in the mesophyll chloroplasts. This starch disappeared from the chloroplasts when the plants subsequently were placed in darkness for 12 hr at 30C. In contrast, accumulated starch did not disappear from the chloroplasts during a 12-hr dark period of 10C (45). In orchardgrass (Dactylis glomerata L.), a temperate grass, the growth of which is not severely retarded by 10C night temperature, there was also an accumulation of starch in chloroplasts during illumination at 30C; however, this starch was mobilized and translocated from the chloroplasts during the dark period even at temperatures as low as 10C (98).

Reduced growth of 'Pangola' may be caused by the failure of starch to be mobilized and translocated from the mesophyll chloroplasts during periods of low night temperature and, as suggested by Hilliard and West (45), this may interfere with photosynthesis. West (97) observed a reduction in the photosynthetic capacity of

plants subjected to low night temperature as evidenced by the reduced ability of chloroplasts from 10C-treated plants to perform the Hill reaction and by the increased packing volume of these organelles. Much evidence now supports the concept that leaf assimilate level is a controlling factor in the rate of photosynthesis (65).

The failure of accumulated carbohydrate to be translocated from the chloroplasts of 'Pangola' may indicate that some temperature-dependent limitation affected starch-degrading enzymes. For this reason, a comparative study of the effect of temperature on amylolytic activities of leaf extracts of 'Pangola' and orchardgrass was undertaken.

Materials and Methods

Plant Materials

'Pangola' plants were propagated from cuttings and potted in 10-cm plastic pots containing a mixture of peat and sterilized (steam-treated) potting soil (1 : 1). Orchardgrass plants propagated by division were potted similarly. All plants were maintained in a greenhouse with a 27C minimum temperature and no supplemental lighting. The plants were watered every other day and received weekly applications of liquid fertilizer. In 'Pangola,' the plant top growth was cut back periodically to approximately 5 cm to maintain the plants in a rapid vegetative growth stage.

Temperature Pretreatments

After 5 weeks of regrowth, 'Pangola' and orchardgrass plants were subjected to two pretreatment regimes for a period of 3 consecutive

days and nights. During the day, all plants received illumination of 3000 ft-c for 12 hrs at 30C, whereas during the 12-hr night period, they were held in growth chambers either at 10C or 30C.

These plants subsequently were used for the preparation of crude enzyme extracts, determination of protein and DNA content, and determination of fresh and dry weights.

Preparation of Enzyme Extracts

Crude enzyme extracts were prepared by grinding 3.0 g of young, fully expanded leaves in an Omni-mixer with 24 ml of ice-cold CaCl_2 (0.001 M). The homogenate was allowed to stand for 1 hr in an ice bath and then centrifuged at $27000 \times g$ for 30 min. The supernatant fraction was passed through Whatman No. 2 filter paper; the filtrate was used immediately as the enzyme source.

Qualitative Determination of Total Amylolytic Activity of 'Pangola'

A modification of the starch-agar plate method of Gates and Simpson (38) was used to determine qualitatively the amylolytic activity of 'Pangola' extracts. The media for starch-agar plates consisted of 2 g agar, 1 g amylose, 0.1 g CaCl_2 , and 0.1 g KH_2PO_4 in 100 ml acetate buffer pH = 5.2. Filter paper was placed on top of jelled media in petri plates, and enzyme extracts were pipetted on the filter paper. Amylolytic activity was measured by the differential iodine staining of media after incubation at 40C for 24 hrs.

Quantitative Measurement of Amylolytic Activity

Amylolytic activity was determined by measuring the rate of disappearance of iodine-staining starch during a period of incubation

with a portion of the crude enzyme extract. The starch substrate for this reaction was prepared by the addition of 5.0 g of soluble starch and 2.7 g KH_2PO_4 to 500 ml of distilled H_2O . The solution was boiled for 1 min, cooled to room temperature and passed through Whatman No. 2 filter paper. Iodine solution was prepared just before each experiment. Five ml of a stock solution (3.0 g KI and 1.27 g I_2 per liter) was diluted to 200 ml with 0.2 M HCl. This diluted solution was used directly in the assay. The enzyme reaction mixture for 'Pangola' contained 4.0 ml of succinate buffer (0.05 M, pH 5.2), 2.0 ml of starch solution and 4.0 ml of the crude enzyme extract. In case of orchardgrass, 6.0 ml of succinate buffer, 2.0 ml of starch solution and 2.0 ml of crude enzyme were added to the 25-ml test tube. Three incubation temperatures were used (incubation being the period of in vitro enzyme-substrate reaction): 10, 20 and 30°C. At a predetermined time, 1.5 ml of aliquots were removed from the reaction test tubes and were mixed with 5.0 ml of I_2 -HCl solution. The absorbance of the starch-iodine complex was determined spectrophotometrically at 625 nm. Appropriate zero-time controls were used so that enzyme activity could be expressed as the change in absorbance during the incubation period.

Protein Determination

One ml 10% TCA (trichloroacetic acid) was added to an equal volume of crude enzyme extract, and the mixture was chilled in an ice bath for 1 hr. The precipitate was separated by centrifuging 20 min at 20000 x g at 0°C. The precipitated protein was hydrolyzed with 1 ml of 1 M NaOH for 24 hr at room temperature. Protein was determined by the method of Lowry et al. (60).

DNA Measurement

Three g of 'Pangola' leaves or 1.5 g of orchardgrass leaves were ground with a 5% sucrose solution containing 0.01 M tris, 0.01 M MgCl_2 , and 0.06 M KCl in an Omni-mixer for 5 min at 0C. The homogenate was centrifuged at 5000 x g and the supernatant passing through a glasswool pad was used for DNA determination.

DNA was determined by a modification (73) of the Burton method (24). To 4 ml of the above leaf homogenate an equal volume of 1.0 M HClO_4 was added, and the mixture after 1 hr in an ice bath was centrifuged 20 min at 27000 x g. After the supernatant was discarded, the precipitate was washed with a mixture of ether, ethanol, and chloroform (2: 2: 1) to remove lipids and chlorophylls. The wash solution was then centrifuged for 20 min at 30000 x g. The precipitates were hydrolyzed with 2 ml 0.6 M KOH for 16-20hr. After this time, 6 ml 0.5 M HClO_4 were added to cause reprecipitation of DNA, and the suspension was centrifuged 20 min at 27000 x g at 0C. After adding 4 ml hot 0.5 M HClO_4 to the precipitate, it was incubated at 70 to 90C for 1 hr. This was centrifuged 20 min at 27000 x g, then 8 ml of diphenylamine reagent were added to 4 ml of supernatant. This was boiled for 15-20 min, then centrifuged at 12000 x g. The absorbance of the supernatant (containing DNA) was determined at 600 nm. Absorbance for each sample was compared to readings of similarly prepared calf thymus DNA standards (24).

Results and Discussion

Qualitative Determination of Starch-degrading Enzyme of 'Pangola'

In these studies, the term "amylolytic activity" is used to refer to the starch breakdown without indicating the involvement of any specific enzyme, for it is not known specifically which starch-degrading enzymes are active in the crude leaf extracts.

Determination of the amylolytic activity by the starch-agar plate method did not disclose differences between extracts from 10C or 30C pretreated plants. Starch hydrolysis was found in the case of both treatments (Fig. 1). This procedure was not sensitive enough to be used to comparing treatments, therefore, subsequent data are from the starch-iodine method of determination. The starch-agar plate method, however, is a simple method which may have application to some worker for screening or selection for breeding.

Effect of Leaf Position on the Amylolytic Activities of Leaf Extracts

In previous studies, much variation was encountered in the starch-degrading activities of leaf extracts among replicates from the same temperature treatment. To determine some of the factors responsible for this variation, the effect of leaf position on the amylolytic activities of leaf extracts was studied. Starting with the first fully expanded leaf at the vegetative shoot apex, the leaves were numbered consecutively down the shoots. The first, second, third and fourth leaves were taken from 10 uniform plants of 5 weeks regrowth at 30C, and crude enzyme extracts were prepared. The highest starch-degrading activity was found in the extracts of

the youngest or uppermost leaves, and this activity decreased as the leaves were taken from progressively lower positions on the shoots (Table 1). This finding is consistent, perhaps, with the idea that upper leaves are in a favored position for the reception of light and thus may produce larger amounts of starch. In addition, those younger leaves are proximate to and supply assimilate for a most active metabolic sink, the rapidly growing apical region of the shoot. The presence of this sink for leaf assimilate would also favor comparatively high rates of carbohydrate production in the uppermost leaves (89). If, indeed, the rates of carbon assimilation in young leaves are sufficiently high to raise the levels of assimilate starch, it is likely that greater amounts of starch-degrading enzymes would also be present.

Data of Table 1 also show the importance of judicious leaf selection in preparing extracts for the determination of amylolytic activity. In the following experiments, amylolytic activities were determined by using leaf samples combining both the first and second fully expanded leaves of uniform shoots.

Effects of Low Temperatures on the Amylolytic Activity

Pretreatment of 'Pangola' at a night temperature of 10C reduced the amylolytic activities of leaf extracts when compared with the activities of extracts from plants receiving 30C nights (Table 1). This reduction in enzyme activity occurred irrespective of the incubation temperature. For incubation temperatures at 10, 20 and 30C, the reductions in amylolytic activity (on dry weight basis) attributable to 10C night pretreatment were 53%, 51% and 47%, respec-

tively. In contrast, pretreatment of orchardgrass at 10C night temperature had no effect on the amylolytic activities of leaf extracts. This was true at all incubation temperatures.

The temperature during enzyme assay had a pronounced effect on enzyme activity irrespective of pretreatment and species. However, the starch-degrading activities of 'Pangola' extracts were reduced to a greater extent by low temperature than the activities of leaf extracts of orchardgrass. In 30C pretreated 'Pangola' changing the incubation temperature from 30C to 10C reduced the amylolytic activity of 'Pangola' extracts by 69%, whereas the activity in orchardgrass extracts were reduced by only 52%. Similar reductions were observed using extracts from 10C pretreated plant material.

Amylolytic activities of the leaf extracts from the various treatments were also calculated on the basis of activity per unit protein (Fig. 2), activity per unit DNA (per cell basis) (Fig. 3), and activity per unit fresh weight (Table 3). In each case, results were the same as those based on activity per unit dry weight (Table 2).

Low night temperatures depress growth in 'Pangola'; this growth depression is accompanied by an accumulation of starch in the mesophyll chloroplasts (45). West (96) stated that accumulation of starch may result from: 1) increased synthesis of starch, 2) decreased activity of starch-degrading enzymes, 3) decreased utilization of the products of starch degradation at metabolic sinks, and/or 4) a decrease in translocation of the products of starch breakdown. In the present work one of these proposed causes, the effect of low night temperature pretreatments on starch-degrading activities of crude leaf extracts from 'Pangola,' has been investigated. Further, as

a comparison, the effects of low night temperature pretreatments on the starch-degrading enzymes of orchardgrass was determined. The effect of temperature treatments during incubation on the starch-degrading activities of leaf extracts of 'Pangola' and orchardgrass was also determined.

Alpha-amylase, beta-amylase, and phosphorylase are generally believed to be the enzymes responsible for starch degradation (3). But there is much confusion as to the localization and compartmentation of these enzymes in relation to their function in the plant cell. It was assumed that in the main. This method measured activities of the amylases. Since preliminary experiments did not demonstrate appreciable phosphorylase activity, assay conditions favorable for amylase activity were used routinely. Both alpha- and beta-amylases have been postulated to have roles in starch degradation in leaf tissue. Haapala (41) attributed the rapid hydrolysis of starch accumulated in chloroplasts of Stellaria media during prolonged illumination to a rapid increase in the activity of beta-amylase. Gates and Simpson (38) found an association between the starch contents and alpha-amylase activities of leaves of a wide range of higher plants. Further, Juliano and Varner (51) suggested that alpha-amylase is the major enzyme involved in the initial degradation of starch of pea cotyledons to more soluble forms and that beta-amylase and phosphorylase assist in the conversion to free sugars.

In brief, it has been shown that 10C night temperature pre-treatment strongly repressed the starch-degrading activity of extracts of 'Pangola' leaves. This was not the case with leaf extracts of orchardgrass. Low temperatures of incubation during enzyme assay decreased

the activities in extracts from leaves of both 'Pangola' and orchard-grass; however, the effect on the tropical species was more pronounced. These results explain, at least in part, the preferential accumulation of starch in the chloroplasts of tropical grasses during periods of low night temperature and, consequently, reduced photosynthesis and growth. West (96) suggested that the accumulation of starch in chloroplasts when plants were subjected to 10C night temperature provided evidence for any of several mechanisms for the influence of low temperatures on growth and photosynthesis: 1) the accumulation of starch allows a concomitant accumulation of certain photosynthetic intermediates which by their presence inhibit further CO_2 fixation, 2) continued accumulation of starch without degradation and transport might fill the chloroplasts to the extent that they are injured physically, thus impairing their function, and 3) the starch grains themselves might actually provide a shading effect on the chloroplasts below, thus preventing efficient light utilization. These results would equally as well explain the resistance of temperate grasses to growth retardation by low night temperatures. In the latter case, starch-degrading enzymes would remain active to produce the soluble sugars which are characteristically found in leaves of temperate plants during periods of depressed temperature.

Table 1. EFFECT OF LEAF POSITION ON THE AMYLOLYTIC ACTIVITIES OF
LEAF EXTRACTS OF FIVE WEEKS REGROWTH OF 'PANGOLA'

Leaf position (apex to base)	AMYLOLYTIC ACTIVITY (40D/g fr. wt./25 min)
1	5.72
2	4.61
3	3.09
4	2.27

The activities of leaf extracts were determined at 30C. Leaves were numbered consecutively from the uppermost fully expanded leaf of the shoot to the base of the shoot. F test shows a significant difference at $P = .01$.

*Each entry in Table 2 is an average of three measurements. Individual values for S_x were calculated for each incubation temperature, and significance within each incubation temperature is shown by letters beside each mean. $S_{\bar{x}}$ for the incubation temperatures means were 0.87 and incubation temperature was found to have a highly significant effect.

**Means of experiments with incubation temperature of 30C having the same letter do not differ at $P = 0.05$ according to Duncan's multiple range test. $S_{\bar{x}} = 2.55$.

Means of experiments with incubation temperature of 20C having the same letter do not differ at $P = 0.05$ according to Duncan's multiple range test. $S_{\bar{x}} = 1.75$.

Means of experiments with incubation temperature of 10C having the same letter do not differ at $P = 0.05$ according to Duncan's multiple range test. $S_{\bar{x}} = 1.56$.

Duncan's multiple range test procedures can be obtained in Steel, R. C. D. and J. M. Torrie, 1960. Principles and Procedures of Statistics. McGraw-Hill, Inc., New York.

Table 2. THE EFFECT OF NIGHT TEMPERATURE PRETREATMENT AND INCUBATION TEMPERATURES ON THE AMYLOLYTIC ACTIVITIES OF LEAF EXTRACTS (ON DRY WEIGHT BASIS)

Enzyme Incubation Temperature C	Grass	Pretreatment Temperature C	Amylolytic Activity* ΔOD/g dry wt/10 min				Pretreatment Mean**	Incubation Mean
			1	Experiment				
				2	3	4		
30	'Pangola'	10	22.85	20.63	19.52	19.06	20.51a	48.64
		30	42.25	45.50	36.45	29.57	38.44b	
	Orchard	10	72.89	70.28	61.83	62.40	66.85c	
		30	72.61	72.44	66.72	63.29	68.77c	
20	'Pangola'	10	11.86	13.46	7.57	8.17	10.27a	32.30
		30	25.67	19.81	21.69	17.14	21.08b	
	Orchard	10	49.61	43.15	44.66	54.25	47.92c	
		30	51.68	50.83	48.80	48.44	49.94c	
10	'Pangola'	10	5.06	5.81	4.23	6.96	5.52a	19.62
		30	11.79	13.06	11.02	11.03	11.73b	
	Orchard	10	26.67	27.68	25.13	33.24	28.18c	
		30	35.34	38.94	28.94	29.33	33.07d	

*Each entry in Table 3 is an average of three measurements. Individual values for $S_{\bar{x}}$ were calculated for each incubation temperature, and significance within each incubation temperature is shown by letters beside each mean. $S_{\bar{x}}$ for the incubation temperatures means were .15 and incubation temperature was found to have a highly significant effect.

**Means of experiments with incubation temperature of 30C having the same letter do not differ at $P = 0.05$ according to Duncan's multiple range test. $S_{\bar{x}} = .42$.

Means of experiments with incubation temperature of 20C having the same letter do not differ at $P = 0.05$ according to Duncan's multiple range test. $S_{\bar{x}} = .32$.

Means of experiments with incubation temperature of 10C having the same letter do not differ at $P = 0.05$ according to Duncan's multiple range test. $S_{\bar{x}} = .26$.

Table 3. THE EFFECT OF NIGHT TEMPERATURE PRETREATMENT AND INCUBATION TEMPERATURES ON THE AMYLOLYTIC ACTIVITIES OF LEAF EXTRACTS (ON FRESH WEIGHT BASIS)

Enzyme Incubation Temperature C	Grass	Pretreatment Temperature C	Amylolytic Activity* AOD/g Fresh wt/10 min				Pretreatment Mean **	Incu- bation Mean
			Experiment					
			1	2	3	4		
30	'Pangola'	10	3.93	3.96	3.69	3.64	3.81a	8.87
		30	7.31	7.28	6.05	5.47	6.53b	
	Orchard	10	13.12	12.72	11.81	11.17	12.21c	5.91
		30	13.87	13.04	13.41	11.33	12.91c	
20	'Pangola'	10	2.04	2.57	1.43	1.56	1.90a	3.59
		30	4.44	3.17	3.60	3.17	3.60b	
	Orchard	10	8.93	7.81	8.53	9.71	8.75	
		30	9.87	9.15	9.81	8.67	9.38	
10	'Pangola'	10	.87	1.11	.80	1.33	1.03a	
		30	2.09	2.09	1.83	2.04	2.00b	
	Orchard	10	4.80	5.01	4.80	5.96	5.14c	
		30	6.75	7.01	5.76	5.25	6.19d	

Figure 1. STARCH-DEGRADING ACTIVITY OF CRUDE EXTRACTS AS SHOWN BY THE STARCH-AGAR
PLATE METHOD.

1. 'Pangola' pretreated at 30C for 3 nights
2. 'Pangola' pretreated at 10C for 3 nights
3. Water
4. Alpha-amylase



Figure 2. THE EFFECT OF LOW TEMPERATURE ON THE AMYLOLYTIC ACTIVITY OF 'PANGOLA' AND ORCHARDGRASS ON PROTEIN BASIS.

'Pangola'

ORCHARDGRASS

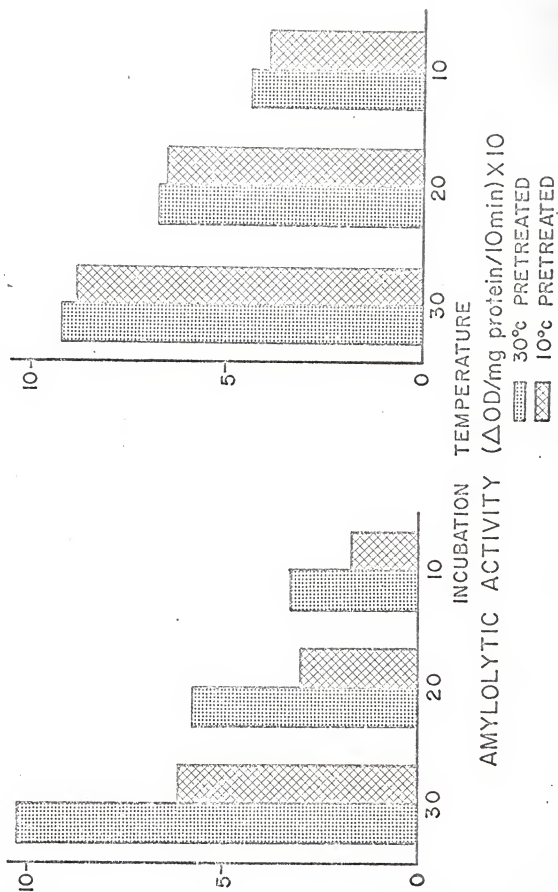
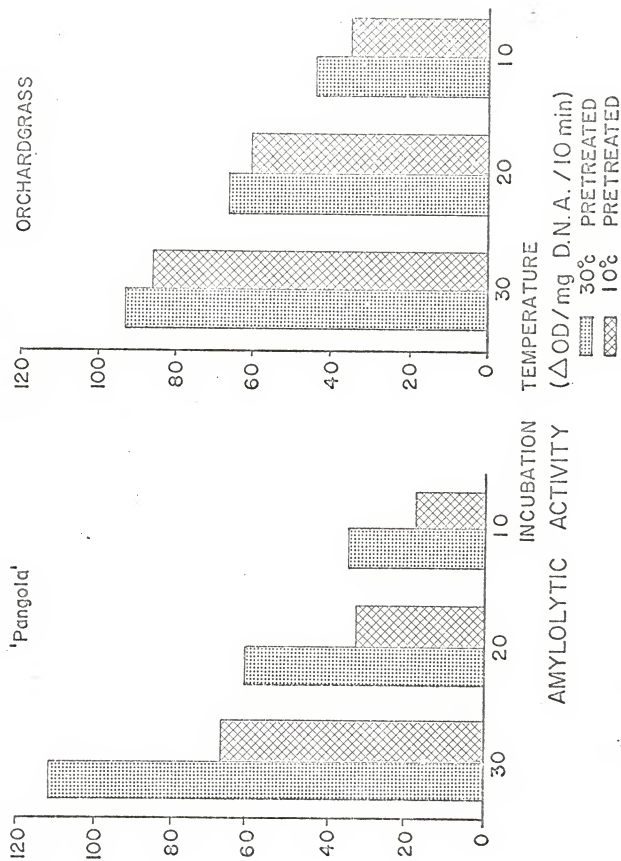


Figure 3. THE EFFECT OF LOW TEMPERATURE ON THE AMYLOLYTIC ACTIVITY OF 'PANGOLA' AND ORCHARDGRASS ON DNA BASIS.



CHAPTER III

REVERSAL OF LOW TEMPERATURE EFFECTS ON A TROPICAL PLANT BY GIBBERELIC ACID

Introduction

Yields of 'Pangola' (Digitaria decumbens Stent.), a tropical pasture grass, are reduced when night temperatures drop to 10C. It has been shown that growth and photosynthesis are reduced by this temperature treatment (95, 96, 97). Further, it was indicated in Chapter II that starch accumulation under these conditions is the result of reduced activity of starch-degrading enzymes. A practice, then, which would increase the amount of activities of these enzymes (thereby increasing both the degradation of assimilate starch and the levels of soluble sugars for transport) could perhaps be used to reverse the effects of low temperature on tropical plants and increase yields at these temperatures.

It has been established that treatment of barley endosperm with gibberellic acid (GA_3) increased the level of alpha-amylase (28, 35, 49, 86). Other workers have shown that GA and other metabolites affect protein and ribonucleic acid synthesis, activities of starch-degrading enzymes, levels of starch, and general growth (6, 13, 37, 51, 53, 58, 100). These reports suggested that GA_3 might be effective in reducing the low temperature retardation of growth of tropical plants by enhancing degradation and utilization of accumulated starch. Therefore, this portion of the study analyzed

the effect of GA_3 on growth, amylolytic activity, and starch degradation in a tropical plant.

Materials and Methods

Plant Materials and Treatments

For the measurement of GA_3 effect on growth, a large number of stem cuttings, each consisting of one internode with upper and lower nodes intact, were grown in sand culture for 5 days in a high humidity chamber. The young plants were then potted in 10 cm plastic pots containing a mixture of peat and sterile potting soil (1:1, v/v) and grown in a greenhouse with no supplemental lighting at a minimum temperature of 27C for 24 days. These plants were watered daily and received weekly applications of liquid fertilizer. Twenty-eight uniform plants were selected and four treatments were applied at random to seven replicates. These treatments included 10C or 30C night temperature regimes of 12 hr with or without application of 10^{-5} M GA_3 (Sigma Chemical Co.). Ten hours before the plants were placed under the 10C or 30C night temperature regimes, the GA_3 solution was applied with a hand-sprayer until all the leaves were thoroughly wetted. Control plants were sprayed with distilled water. The plants were grown for an additional 15 days in growth chambers providing 30C day temperatures with 3000 ft-c illumination and the two night temperature regimes, after which their fresh weights, dry weights, leaf areas, and increases in shoot lengths were measured.

For the measurement of amylolytic activity and starch content, plants were selected from large populations initially propagated from

cuttings and allowed to grow under greenhouse conditions as described above. Periodically, the top growth was removed to 5 cm above soil level to maintain the plants in a rapid growth stage. Plants of 4 to 6 weeks of regrowth were used to determine the effects of GA_3 and low night temperature regimes on amylolytic activity and starch content. For all experiments, each of four treatments was applied to three replicate plants. These treatments included 10C or 30C night regimes with or without application of 10^{-5} M GA_3 . Ten hours before imposition of the night temperature regimes, the GA_3 solution was applied as above. Plants were held in growth chambers at 30C with 3000 ft-c illumination during the 12-hr day, and temperature treatments were continued for three consecutive days and nights before the measurements of starch content and amylolytic activity were made.

Preparation of Enzyme Extracts and Measurement of Amylolytic Activity

Crude enzyme extracts were prepared and amylolytic activity was measured using the method described in Chapter II.

Preparation of Leaf Samples and Measurement of Starch

Young, fully expanded leaves (approximately 3 cm excised from either end) were cut into 5-10 mm lengths and mixed thoroughly, then weighed out in 100 mg samples. Each freshly cut sample was dropped immediately into 15 ml boiling 80% ethanol, boiled for 30 seconds, and removed from the heat. The sample was allowed to extract at room temperature for 1 hr after which the alcohol was decanted and discarded. At least three such 1-hr extractions

were performed on each sample to remove the soluble sugars and pigments. At the completion of extraction, leaf samples were placed in a 70C drying oven for 72 hr. After drying, the samples were weighed and placed in a glass homogenizer with 5.0 ml 0.1 M acetate buffer (pH 4.8) and ground approximately 5 min. Five ml more of the grinding solution were used to wash the homogenate into a 25-ml Erlenmeyer flask. An equivalent of 20 mg amyloglucosidase (from *Rhizopus*, Sigma Chemical Co., 1200-3000 units/s) were added to each flask and the flasks stoppered lightly and placed in a 55C - 60C water bath on a shaker for 90 min. At the end of incubation, the flask contents were centrifuged at 27000 x g for 30 min. Aliquots of the supernatant fraction were used for measurement of glucose.

Glucose content was measured by the glucose oxidase method (Glucostat, Worthington Biochemical Corp., Freehold, N. J.) and the mg glucose per g fresh weight of tissue were converted to mg starch per g fresh weight of tissue.

Results and Discussion

Preliminary Results on the Effect of GA on Growth of 'Pangola'

The effect of GA_3 under 10C or 30C temperature regimes was determined. Many different concentrations of GA_3 were used on 'Pangola' of different ages and for different treatment periods. From experiments performed 10^{-5} M GA_3 seemed to be the best concentration for the age of plants that were used in this investigation, so all subsequent experiments received applications of 10^{-5} M GA_3 .

Growth

The application of gibberellic acid (GA_3) completely prevented the depression in growth of plants exposed to 10C night temperature (Table 4). In both the control plants (30C night) and the GA_3 treated 10C plants, there were highly significant increases in dry weight, total

leaf area, and shoot length. Although GA_3 prevented growth depression in plants subjected to 10C night temperature, it had no effect in enhancing the growth of plants subjected to 30C nights. These findings are consistent with those of Blacklow and McGuire (13), who demonstrated that a foliar spray of 1% GA_3 caused an increase in foliage growth of tall fescue varieties in the field during the winter and with other workers (5, 100) who found that applications of GA_3 increased regrowth and new growth in winter pastures.

Amylolytic Activity

Amylolytic activity of plants grown in 10C nights was reduced below the level of that of plants grown in 30C nights, but was restored by GA_3 treatment (Table 5). However, GA_3 treatment had no effect on the starch-degrading activities of leaf extracts from plants treated at 30C night temperatures. These data corroborate field work in which the total and specific activities of alpha-amylase and invertase in the leaves of tobacco were increased by application of GA_3 (58), as well as other reports that GA_3 increased the levels of alpha-amylase in higher plants (28, 35, 49, 86).

Effect of GA_3 on in Vitro Enzyme Activity

To determine if GA_3 increased amylolytic activity by direct participation in the reaction or whether its action was dependent on the activity of the functional, intact cell GA_3 was added directly to the reaction mixtures containing the crude enzyme extracts from 30C-treated plants. No increases were found in the amylolytic activity of 'Pangola' extracts to which GA_3 was added (Table 5). This was true at both the 10C and the 30C incubation temperatures. This indicates that GA_3 does not exercise its effect through some direct effect on the enzyme or enzyme-substrate complex. It is possible that the GA_3 effect reflects an increase in protein synthesis,

particularly enzymes responsible for carbohydrate mobilization. This is in accord with and supported by reports that GA_3 can regulate protein and ribonucleic acid synthesis (5, 8, 37, 50).

Starch Content

Once it was established that application of GA_3 increased amylolytic activity in the leaf as well as increasing plant growth, the starch content of the plants after exposure to different temperatures and treatments was determined (Table 5). There was little starch found in plants grown at 30C with or without application of GA_3 , while the greatest amount of starch was found in the plants grown at 10C nights without GA_3 applications. More than 50% of the starch that accumulated as a result of low night temperature was mobilized and made available for growth by the GA_3 treatment, apparently as a result of increases in the activity of the starch-degrading enzymes. The increased amylolytic activity in GA_3 -treated plants grown at 10C nights apparently provided for the conversion of 5 mg more starch per g fresh weight leaf tissue to soluble sugars used in growth than in the case of plants grown at 10C and not treated with GA_3 .

The data reported here suggest that treatment of 'Pangola' with GA_3 may be an excellent approach to extending pasture productivity later into the growing season. However, further studies dealing with the optimum time for application and concentrations of GA_3 required to achieve the desired results should be made on a field trial basis.

Table 4. EFFECT OF LOW NIGHT TEMPERATURE AND GIBBERELLIC ACID ON THE GROWTH OF 'PANGOLA'

Growth per Plant	Treatments*			
	10C	10C + GA ₃	30C	30C + GA ₃
dry weight (g)	1.04 ^a	1.51 ^b	1.46 ^b	1.39 ^b
leaf area (cm ²)	39.4 ^a	79.7 ^b	80.6 ^b	97.9 ^b
shoot length (cm)				
before treatment	27	29	34	30
after treatment	40	71	103	91
differences	13 ^a	42 ^b	69 ^c	61 ^c

* Means of the treatments having the same letter are not significantly different at P = 0.01 according to the F test. Each value is the mean of seven replicates of one experiment.

Table 5. EFFECT OF GIBBERELIC ACID ON AMYLOLYTIC ACTIVITY AND STARCH CONTENT OF 'PANGOLA' AT TWO NIGHT TEMPERATURE REGIMES

Treatment	Amylolytic Activity* (OD/g Dry wt/10 Min)	Starch Content** (Mg/g Fresh Wt.)
10C	19.23 ^a	8.19 ^a
10C + GA ₃	28.85 ^b	3.45 ^b
30C	33.91 ^{b c}	0.64 ^c
30C + GA ₃	35.66 ^c	0.44 ^c

* Each value is the mean of eleven experiments. Means having the same letter do not differ at $P = 0.05$ according to Duncan's multiple range test. $S_{\bar{x}} = 2.15$.

** Each value is the mean of six experiments. Means having the same letter do not differ at $P = 0.05$ according the Duncan's multiple range test. $S_{\bar{x}} = 0.918$.

Table 6. EFFECT OF GIBBERELIC ACID ON THE IN VITRO ACTIVITY OF THE STARCH-DEGRADING ENZYMES OF 'PANGOLA'

Incubation Temperature	Amylolytic Activity* (Δ OD/g Dry wt/10 Min)	
	Control	GA ₃ Added
30C	29.63 ^a	28.04 ^a
10C	7.36 ^b	7.79 ^b

* Means of three experiments, each having three replicates. Values having the same letter are not significantly different at the 0.05 level according to the F test.

CHAPTER IV
LOW NIGHT TEMPERATURE, GROWTH AND
AMYLOLYTIC ACTIVITY OF TWO SPECIES OF DIGITGRASS

Introduction

'Pangola' (Digitaria decumbens Stent.) exhibited a marked reduction in growth and photosynthesis when subjected to low night temperature. Evidence obtained in Chapters II and III showed that low night temperatures repress the mobilization of assimilate starch from 'Pangola' chloroplasts by reducing the activities of amylolytic enzymes, but had no effect on a temperate species, orchardgrass. In the nursery collection of the University of Florida there are different species of digitgrass which exhibit different degrees of winter survival (71). It was important and interesting to see if there was any direct relation to the effect shown here which low temperatures have on 'Pangola' and winter survival of digitgrasses. Therefore, the growth and amylolytic activities of the two digitgrasses, Digitaria pentzii (P.I. 299753), which has a high winter survival rate and Digitaria decumbens Stent., which has a low winter survival rate, were investigated.

Materials and Methods

Plant Material

Plants of 'Pangola' and Digitaria pentzii were established

from cuttings by direct rooting in 10-cm plastic pots containing a mixture of sandy loam and peat (3:1). These plants were held in a greenhouse with a 30C minimum temperature for 4 weeks until subjected to the temperature regimes of 10 or 30C for 3 consecutive 12-hr nights and 30C during the day. All plants received weekly applications of a complete liquid fertilizer and were watered every other day. Periodically, the plants were cut back to 5 cm in order to maintain a rapid vegetative growth stage.

Preparation of Enzyme Extracts and Measurement of Amylolytic Activity

Crude enzyme extracts were prepared and amylolytic activity measured by the methods described in Chapter II.

Results and Discussion

Effects of Low Night Temperature on the Amylolytic Activities of Two Species of Digitgrass

The amylolytic activities of leaf samples of the two species were determined. The amylolytic activities of leaf extracts were higher in all experiments and species when the plants received 30C night temperature than when the plants received 10C night temperature (Table 7). Average reductions of amylolytic activity in 'Pangola' and *D. pentzii* as a result of low-night-temperature treatment were approximately the same, 18 and 23 per cent, respectively.

Growth of Two Species of Digitgrass Under Different Temperature Regimes

Plants of both species were established by growing cuttings

in sand for a week at 13C-nights and 24C-days, followed by a period of 3 weeks under the three temperature regimes given in Table 8. Seven plants of each species were used for each treatment. After the third week, the plants were harvested at ground level, and the fresh and dry weights of the tops were determined. 'Pangola' was found to have a higher growth rate under all temperature regimes than D. pentzii (Table 8); however, the growth rates of both species were reduced similarly by lowering the temperature.

Although D. pentzii displays more winter-hardiness than D. decumbens ('Pangola'), the latter species appears to be somewhat more productive under the low temperature regimes used in this study. It is apparent, however, that productivity may be severely limited in both species as a result of low temperatures. Chapter II previously indicated that growth retardation in 'Pangola' as a result of low night temperatures (ca. 10C) may be explained in part by a reduction in the activities of starch-mobilizing enzymes. It has been shown in this study that the amylolytic activities of leaf extracts of D. pentzii are also depressed as a result of low-night-temperature treatments, much the same as in the case of 'Pangola.' It is quite possible therefore that reduced amylolytic activity in leaves may also explain in part the growth reduction of D. pentzii by low night temperature.

Table 7. THE EFFECT OF NIGHT TEMPERATURE PRETREATMENT ON THE AMYLOLYTIC ACTIVITIES OF LEAF EXTRACTS OF TWO SPECIES OF DIGITGRASS

	Pretreatment temperature	AMYLOLYTIC ACTIVITY ($\Delta OD/mg$ protein/20 min)			
		Experiments			
		C	1	2	3
'Pangola'	10	0.33	0.28	0.25	0.29
	30	0.41	0.36	0.28	0.35
<u>Digitaria</u> <u>pentzii</u>	10	0.44	0.27	0.23	0.32
	30	0.58	0.35	0.29	0.41

Plants received the pretreatment temperatures of 10 or 30C during the 12-hr night period while, in all cases, the day temperature was 30C. Amylolytic activity of the extracts was measured at 30C.

Table 8. THE EFFECT OF VARIOUS TEMPERATURE REGIMES ON THE GROWTH OF TWO SPECIES OF DIGITGRASS

Temperature Regime (C night/day)	GROWTH			
	Fr. wt. (g)		Dry wt. (mg)	
	'Pangola'	<u>D. pentzii</u>	'Pangola'	<u>D. pentzii</u>
21/32	5.02	3.92	1395	1168
13/24	2.50	1.45	747	492
4/16	0.97	0.54	299	155

Low temperature highly significantly reduced the fresh weight and dry weight of both species at $P = 0.01$.

CHAPTER V
SUMMARY AND CONCLUSIONS

Growth, amylolytic activity, and effect of gibberellic acid (GA_3) on growth and certain biochemical events in 'Pangola' (*Digitaria decumbens* Stent.) at low temperatures were determined. Crude extracts were made from leaves of 'Pangola' taken from consecutive positions along single tillers and the amylolytic activities were determined. Enzyme activity varied greatly with the position of the leaves on the shoots. Young, fully expanded leaves near the shoot apices have the highest starch-degrading activity. This activity decreased in extracts of older leaves taken from progressively lower positions on the shoots. Activities of leaf extracts of 'Pangola' were compared to those of orchardgrass (*Dactylis glomerata*L.) subjected to the same treatments. Treatment of 'Pangola', a tropical species, to 10C night temperature for three nights markedly reduced the starch degrading enzyme activity of leaf extracts, whereas this was not the case with orchardgrass, a temperate species, the growth of which is little affected by 10C night treatments.

In addition to 10C vs. 30C night effect on starch degrading enzymes, significant decreases in dry weight, leaf area, and shoot length in 'Pangola' occurred under 10C night temperature compared to 30C treatment. Also, starch accumulated in 'Pangola' leaves pretreated in 10C night, while little starch was accumulated at 30C night temperature.

Application of GA_3 reversed the effect of low night temperature on the growth of 'Pangola.' This was evidenced by increases in dry weight, leaf area, and shoot length. In addition, treatment with GA_3 increased the amylolytic activity in crude leaf extracts and decreased the level of accumulated starch in leaves of plants subjected to 10C for three nights compared to plants subjected to 10C nights without GA_3 treatments.

Direct addition of GA_3 to the reaction mixture did not alter in vitro amylolytic activity of crude leaf extracts of 'Pangola' during incubation at 10C or 30C. This observation supports the idea that low temperature effects were due to decreased synthesis of amylolytic enzymes, not allosteric interaction of GA_3 with the enzyme.

A comparison was made of the growth and amylolytic activities of leaf extracts of 'Pangola' and Digitaria pentzii (PI 299753) at low temperatures. D. pentzii exhibits better winter survival (as distinguished from the effects of low temperature) than does 'Pangola.' The reduction in the growth and amylolytic activity caused by low temperatures are comparable in the two digitgrass species.

Data reported in these investigations suggest that reduction of growth of 'Pangola' by suboptimal night temperatures is due to depression of amylolytic activity. It also shows that there is no relation between winter survival and amylolytic activity. Moreover, the data suggest that treatment of 'Pangola' with GA_3 may be a good way to extend

pasture productivity later into the growing season. However, further studies in the field are required to at least determine the optimum application time and concentrations of GA_3 required to achieve desired results before it can be recommended for commercial use.

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